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Quantitative Aspects of Risk Assessment in Chemical Carcinogenesis

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Quantitative Aspects of Risk Assessment in Chemical Carcinogenesis
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Formation and Subsequent Repair of Alkylation Lesions in Tissues of Rodents Treated with Nitrosamines*

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Abstract. There is evidence that the formation of O^6-alkylguanine in DNA may be an important reaction in the induction of tumors by dimethylnitrosamine and related carcinogens. The removal of this product from DNA may protect against carcinogenesis. The removal process has been studied both in vivo after administration of labelled dimethylnitrosamine and in vitro by incubation of isolated enzyme preparations with alkylated DNA. It has been found that:

1. Activity removing O^6-alkylguanine from DNA is present in a number of tissues, but is most active in liver.
2. High doses of dimethylnitrosamine (20 mg/kg) inhibit removal of O^6-methylguanine from DNA in vivo completely in rat kidney and hamster liver and partially in rat liver. This loss of activity was also seen in extracts prepared from these tissues assayed in vitro.
3. Chronic exposure to low levels of dimethylnitrosamine led to an increase in the activity of the hepatic enzyme removing O^6-methylguanine.
4. Hypophysectomy or thyroidectomy decreased the activity of the hepatic removal system for O^6-methylguanine and treatment with growth hormone or thyroxin increased it.
5. Dimethyl- and diethylnitrosamine are rapidly absorbed from the upper part of the small intestine (but not from the stomach). The degree of alkylation observed in the liver was independent of whether the carcinogen was administered orally or by i.v. injection, but at doses of 0.1 mg/kg or less, the reaction with the kidney was much lower after oral administration. Therefore, at low oral exposures to the carcinogen, the liver, which has the greatest capacity to remove O^6-methylguanine from its DNA receives a relatively greater proportion of the alkylation.

These results are discussed in terms of the degree to which the DNA repair system removing O^6-alkylguanine might contribute towards a threshold dose and that variations in this activity might be reflected in the magnitude of this threshold.

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Introduction

N-nitroso-compounds form an important class of carcinogenic chemicals which may be of environmental importance (Druckrey et al., 1967; Magee et al., 1976; IARC, 1978). The mechanism by which these carcinogens initiate neoplastic growth is not well understood but there is substantial evidence favoring the hypothesis that the simple dialkylnitrosamines and alkylnitrosamides act by means of their conversion to alkylating agents. Alkylating species are generated from the nitrosamines by metabolic activation and from the nitrosamides by chemical decomposition at physiological pH. It is widely believed that DNA is the critical alkylation target. Alkylation takes place at at least 12 sites within the DNA molecule (Lawley, 1976; Singer, 1976; Pegg, 1977a). Although alkylation at the 7-position of guanine is the most extensive reaction with the DNA bases and provides a reliable measurement of the degree of interaction with the carcinogen (Swann and Magee, 1968, 1971), recent experiments have suggested that alkylation of oxygen atoms may be the critical reaction (Goth and Rajewsky, 1974; Margison and Kleihues, 1975; Nicoll et al., 1975; Pegg, 1977a). These laboratories have observed a correlation between the production and persistence of O^6-alkylguanine and the occurrence of tumors in a variety of organs of rodents exposed to N-nitroso-carcinogens. It has, therefore, been suggested that the ability of tissues to catalyze the removal of O^6-alkylguanine from DNA may provide a protective mechanism against carcinogenesis. A race between this activity and cellular DNA synthesis may determine whether the alkylation might have the chance to lead to a neoplastic cell. Therefore, factors influencing the activity of this enzyme system may be of importance in determining sensitivity to cancer induction by the nitrosamines. For this reason, a detailed investigation of the level and regulation of this activity in various rodent tissues has been made. These studies have been carried out in vivo by following the loss of radioactive O^6-alkylguanine from DNA after treatment with labelled nitrosamines and in vitro by studying the ability of tissue extracts to catalyze the removal of O^6-alkylguanine from an alkylated DNA substrate.

Methods

Labelled N-nitroso-compounds were purchased from New England Nuclear Corporation, Boston, MA, USA and diluted to the required specific activity by addition of purified unlabelled material. Female Sprague-Dawley strain rats (intact, hypophysectomized and thyroidectomized) and male Syrian golden hamsters were purchased from Charles River Breeding Laboratories, Wilmington, MA, USA. Nitrosamines were administered orally as solutions in water or by intraperitoneal or intravenous injections of solutions in 0.9% (w/v) NaCl at about 10.00 a.m. DNA was isolated and analyzed as previously described (Pegg, 1977b; Pegg and Hui, 1978a, b). Tissue extracts capable of catalyzing the removal of O^6-methylguanine from DNA were prepared as described by Pegg (1978a, b).

Dimethylnitrosamine uptake from stomach or intestine was measured as follows. The stomach was ligated at the pyloric sphincter and 10 ml of water containing 0.2 μg [^{3}H]dimethylnitrosamine or 1 μg [^{14}C]diethylnitrosamine (about 50,000 cpm.) was introduced. Samples were removed at various times later and the radioactivity re-

maining determined. [^{14}C] or [^{3}H]Sorbitol (as appropriate) was also placed in the solution to provide a labelled molecule which was not absorbed and could be used to correct for changes in the volume of gastric fluid. Similar experiments were carried out using a portion of small intestine from the pylorus to about 15 cm further down which was ligated at each end. In this case, individual rats were used for each point and the entire contents were assayed for radioactivity at various times after introduction of 1 ml of 0.9% NaCl containing the nitrosamines.

Results

1. Organotropism of Repair

Previous studies in a number of laboratories have confirmed and extended the original finding of Goth and Rajewsky (1974) that the liver appears to be the most active tissue in catalyzing removal of O^6-alkylguanine from its DNA and that the brain is among the least active (reviewed, Pegg, 1977a). All of these experiments were carried out by administration of a radioactive carcinogen followed by measurements of the time course of loss of the labelled purine from DNA. Recently, we have been able to assay an activity causing removal of O^6-methylguanine or O^6-ethylguanine from acid-precipitable DNA during incubation with alkylated DNA in vitro (Pegg and Hui, 1978a; Pegg, 1978a, b). This activity was not due to a non-specific nuclease since neither 7-methylguanine nor guanine were released from the DNA in a perchloric acid soluble form. Table 1 shows a comparison of the activity of this enzyme in extracts from various tissues from the rat. A small amount of activity was detected in all the extracts; but kidney, and particularly liver, were the most active. Extracts from brain were virtually inactive. As shown in Table 1, these results were unaltered when the substrate was changed from methylated to ethylated DNA. Therefore, the activity which is measured

Table 1. Activity of enzyme catalyzing removal of O^6-alkylguanine from DNA in various tissue extracts

Species	Tissue	Activity (as percentage of that present in rat liver)	
		O^6-methylguanine	O^6-ethylguanine
Rat	Liver	100	100
Rat	Kidney	37	33
Rat	Small intestine	9	a
Rat	Colon	8	a
Rat	Stomach	8	a
Rat	Lung	5	a
Rat	Brain	4	2
Hamster	Liver	78	a
Hamster	Lung	11	a

[a] Not determined. Results were expressed as a percentage of the activity found in rat liver extracts which were able to remove about 150 fmol of O^6-methylguanine or O^6-ethylguanine per mg of protein per hour when incubated with 0.5 mM (as phosphate) DNA substrate containing about 20 μmol of the alkylated base per mol of guanine

in these experiments correlates well with the in vivo measurements. Both experiments indicate the need for estimates of DNA repair in the actual target cells of chemical carcinogens rather than extrapolations from measurements made in cultured lymphocytes or fibroblasts which may express quite different levels of activity.

2. *Capacity of Repair*

The presence of an active repair system for O^6-methylguanine in rat liver leads to significant removal of this product from the hepatic DNA within a short time period after exposure to the carcinogen. This removal is indicated in Figure 1 in which the actual O^6-methylguanine levels found 4 and 24 h after administration of dimethylnitrosamine and the expected initial value calculated from the amount of 7-methylguanine observed are shown. It can be seen that at low levels of exposure, more than 80% of the O^6-methylguanine is removed within 4 h and that by 24 h, about 90% or more is lost after doses of 1 mg/kg or less. It is clear from Figure 1 that removal of O^6-methylguanine from hepatic DNA in the rat is more efficient after lower levels of exposure, but removal does take place after higher doses. As shown in Table 2, this contrasts with removal of O^6-methylguanine from DNA in the rat kidney and in the hamster liver. In these organs, O^6-methylguanine loss occurs at a quite rapid rate after low level exposure (Pegg and Hui, 1978a; Stumpf et al., 1979), but appears to be completely inhibited within a short period after exposure to higher doses. This inhibition is also observed when enzyme preparations removing O^6-alkylguanine from DNA in vitro are examined (Table 2, last column). At 24 h after administration of 20 mg/kg doses of dimethylnitrosamine this activity was barely detectable in rat kidney or liver and in hamster liver. The loss of this activity, therefore, correlates well with the lack of removal of O^6-methylguanine from DNA in vivo from the hamster liver and rat kidney. This suggests that the enzyme assayed in these tissue extracts represents the major

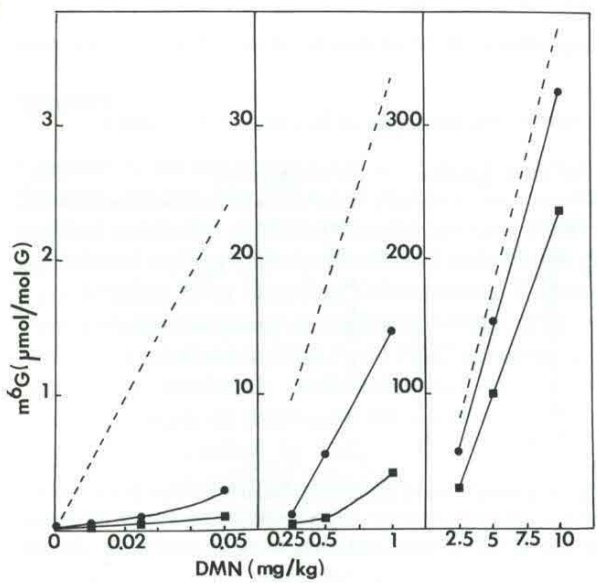

Fig. 1. Content of O^6-methylguanine in rat liver DNA after various doses of dimethylnitrosamine. Rats were given intraperitoneal of [^{3}H] or [^{14}C]dimethylnitrosamine at the doses shown and the O^6-methylguanine (m^6G) present in liver DNA 4 h (●—●) or 24 h (■—■) later was determined (see Methods). The dashed line shows the initial amount of O^6-methylguanine formed as calculated from the amount of 7-methylguanine present (see Pegg and Hui, 1978a)

Table 2. Apparent inhibition or saturation of O^6-methylguanine repair following exposure to high doses of dimethylnitrosamine

Organ	Dose of Dimethylnitrosamine (mg/kg)	Time (h)	O^6-methylguanine present in DNA (μmol/mol guanine)	Activity of enzyme removing O^6-methylguanine from DNA in vitro (fmol/mg/h)
Hamster Liver	0	0	–	113 (100%)
Hamster Liver	20	5	471	–
Hamster Liver	20	24	464	4 (3%)
Rat Kidney	0	0	–	50 (100%)
Rat Kidney	20	24	59	2 (4%)
Rat Kidney	20	48	57	–
Rat Liver	0	0	–	135 (100%)
Rat Liver	20	24	447	15 (11%)
Rat Liver	20	48	305	–

Enzyme activity was measured using extracts prepared from the tissues shown as described by Pegg (1978a) and incubation with DNA substrate as in Table 1. The activity in the tissue from animals not treated with the nitrosamine was designated 100% and the % activity remaining after nitrosamine treatment is shown in parenthesis. The O^6-methylguanine content of tissue DNA was determined at the time shown after injection of 20 mg/kg [^{14}C]dimethylnitrosamine as described by Pegg and Hui (1978a)

part, if not all, of the activity responsible for this reaction in vivo. In the rat liver removal still occurs in vivo despite an almost complete inhibition of the activity measured in vitro suggesting that a second removal pathway must also occur in this tissue. However, even in rat liver the efficiency of removal of O^6-alkylguanine from DNA in vivo is substantially reduced by pretreatment with high doses of dialkylnitrosamines or nitrosamides (Kleihues and Margison, 1976; Pegg, 1978c).

3. *Species Specificity of Removal*

Extensive comparisons of the abilities of extracts from different species to carry out the removal of O^6-methylguanine from DNA have not yet been carried out, but it is evident from the results shown in Tables 1 and 2 that there are considerable differences between the rat and the hamster, particularly in respect to the inhibition of removal activity in the liver at higher doses of dimethylnitrosamine. A more detailed investigation of this phenomenon has recently been published (Stumpf et al., 1979) showing that within a 24 h period, the rat liver can remove more than 50% of the O^6-methylguanine following a dose of 2.5 mg/kg, whereas the hamster liver is able to produce this degree of removal only up to doses of 0.25 mg/kg. It is clear that further species comparisons including those with enzymes isolated from human tissues are urgently required.

4. *Induction of Removal*

Although as described above, large doses of dimethylnitrosamine inhibit removal of O^6-alkylguanine from DNA, pretreatment for several weeks with low doses of the carcinogen appear to induce the activity. Figure 2 shows the time course of removal of

labelled O^6-methylguanine produced in rat liver by injection of 2 mg/kg doses of [^{14}C]dimethylnitrosamine in control rats and rats exposed to daily doses of unlabeled 0.75 mg/kg dimethylnitrosamine for 35 days. It can be seen that at all times, the labelled O^6-methylguanine was less in the treated rats suggesting that the removal pathway had been induced in these animals. There was no difference in the amount of 7-methylguanine or 3-methyladenine found in the DNA at any time showing that activation of the carcinogen to the alkylating species was not affected by the pretreatment (data not shown). In fact, the increase in ability to remove O^6-methylguanine from DNA is probably underestimated by the comparison in Figure 2, since the liver DNA of the pretreated rat contains unlabelled O^6-methylguanine which is not taken into account. As shown in Table 3, the induction of increased ability to catalyze removal of O^6-methylguanine from DNA by pretreatment with dimethylnitrosamine could also be demonstrated in vitro. Extracts from the carcinogen-treated rats were considerably more active in carrying out this reaction on an alkylated DNA substrate than those from control tissues.

At present, the maximal extent to which the activity can be induced or the minimum time and dose of exposure needed to produce an increase have not been determined.

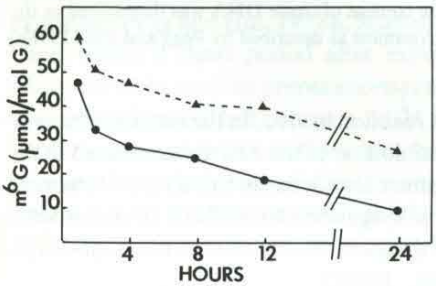

Fig. 2. Effect of pretreatment with dimethylnitrosamine on loss of O^6-methylguanine from hepatic DNA. Control rats (▲ - - - ▲) or rats pretreated with daily doses of 0.75 mg/kg dimethylnitrosamine for 35 days (●——●) were given a 2 mg/kg dose of [^{14}C]dimethylnitrosamine and killed at the times shown. The amounts of O^6-methylguanine (m^6G) present in the hepatic DNA are shown. [There was no significant difference between the content of labelled 7-methylguanine in the two groups at any time. For example, at 2 h the control DNA contained 607 and the pretreated DNA 595 μmol/mol of 7-methylguanine. By 24 h these values had declined to 506 and 496 respectively]

Table 3. Induction of activity removing O^6-methylguanine from DNA in livers of rats treated with dimethylnitrosamine

Pretreatment	Activity removing O^6-methylguanine from DNA (fmol/mg/h)
None	91
0.75 mg dimethylnitrosamine per kg per day for 44 days	285
2 mg dimethylnitrosamine per kg per day for 25 days	233

Enzyme extracts were prepared 24 h after the final administration of dimethylnitrosamine. Details of the preparation of extracts and the assay of activity are given in Table 2

However, similar increases to those seen in the present experiments have been measured in vivo by Montesano et al. (1979a). A dose response curve showing that pretreatment with 0.2 mg/kg daily doses of dimethylnitrosamine for 44 days produces an induction, but that greater doses up to 2 mg/kg produce a larger effect has now been obtained (Montesano et al., 1980b).

5. *Endocrine Effects on Removal*

Hypophysectomy or thyroidectomy of rats prior to administration of dimethylnitrosamine leads to an increase in the amount of O^6-methylguanine found in the liver DNA 24 h after administration of dimethylnitrosamine (Table 4). The amount of 7-methylguanine was only slightly different showing that activation of the carcinogen and interaction with DNA was virtually the same in the control and the hormone-deficient animals. The greater amount of O^6-methylguanine is, therefore, due to a reduction in the rate of removal of this product from DNA. Treatment of hypophysectomized rats with growth hormone partially abolished the effect, but removal of O^6-methylguanine was still less than controls (Table 3). More details of the results in hypophysectomized rats have been published recently (Pegg et al., 1978). However, as hypophysectomized rats are substantially thyroidectomized owing to the loss of thyrotropin, the fact that O^6-methylguanine removal was reduced by thyroidectomy and substantially reversed by thyroxin treatment (Table 3) suggests that a major part of the effect of hypophysectomy may be mediated in this way. If the hypothesis that persistence of O^6-methylguanine in DNA throughout DNA replication could initiate tumors is correct, these endocrine manipulations might change tumor incidence after dimethylnitrosamine administration. Although one such study was inconclusive because tumors were produced in all animals, both control and hypophysectomized

Table 4. Methylated guanine levels present in hepatic DNA of control, hypophysectomized and thyroidectomized rats after treatment with dimethylnitrosamine

Endocrine status	Methylated guanines present in liver DNA (μmol/mol guanine)	
	7-methylguanine	O^6-methylguanine
Control	240	4.3
Hypophysectomized	270	14.2
Hypophysectomized plus growth hormone treatment	276	8.1
Thyroidectomized	230	10.3
Thyroidectomized plus thyroxin treatment	204	5.2

All rats were injected with [^{3}H]dimethylnitrosamine (1 mg/kg) 24 h before death and the content of alkylated purines present in the DNA was determined. Treatment with growth hormone consisted of six daily injections of 1 mg/kg human growth hormone (NIH-GM-B18, which was obtained through the pituitary hormone distribution program of the NIAMD, Bethesda, MD, USA). Treatment with thyroxin consisted of 12 daily injections of 50 μg/kg. The final hormone injection was given immediately prior to the dimethylnitrosamine. Both hormone treatments resulted in significant growth of the treated rats as measured by increased body weight

(Goodall, 1968), it was reported that liver tumors were produced more readily in thyroidectomized rats than controls after single doses of dimethylnitrosamine (Noronha and Goodall, 1977).

6. Effect of Route of Administration on Reaction of Dimethylnitrosamine with Liver and Kidney and Subsequent Repair of Alkylated DNA

After i.v. administration of dimethylnitrosamine, alkylation of liver and kidney DNA could be detected at all doses of dimethylnitrosamine from 1 μg/kg to 10 mg/kg. As measured by alkylation of DNA at the 7-position of guanine 6 h after injection of the

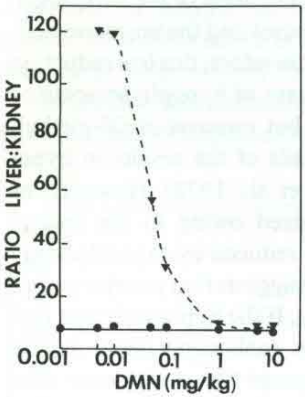

Fig. 3. Ratio of alkylation of DNA in liver to that in kidney after oral or intravenous administration of dimethylnitrosamine. [^{3}H]dimethylnitrosamine was injected i.v. as a solution in 0.9% NaCl into the tail vein (●—●) or placed in the stomach by administration through a feeding tube of 5 ml water containing the nitrosamine (▲- - -▲). The rats were killed 6 h later and alkylation of DNA as measured by formation of 7-methylguanine was determined

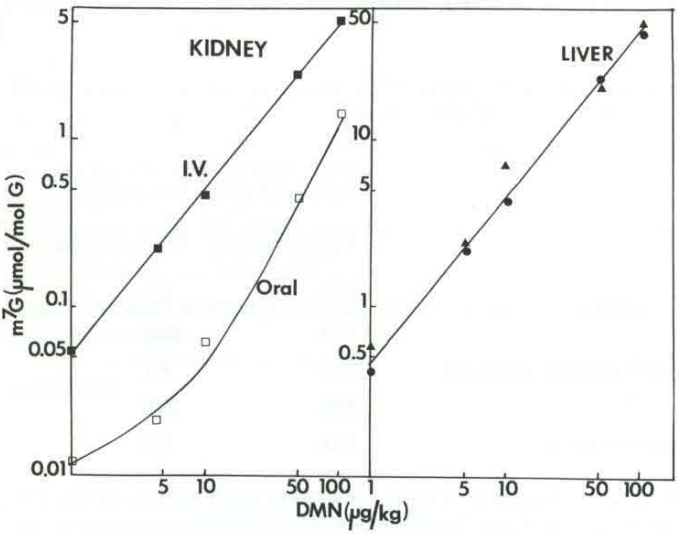

Fig. 4. Presence of 7-methylguanine in DNA of kidney (left panel) and liver (right panel) after intravenous or oral administration of dimethylnitrosamine. Rats were treated and DNA analyzed as described in Figure 3. Results are shown for 7-methylguanine (m^{7}G) present in DNA 6 h after treatment with dimethylnitrosamine by oral administration (□—□, kidney; ▲—▲, liver) or i.v. injection (■—■, kidney; ●—●, liver)

carcinogen, the ratio of reaction with the liver to that with the kidney was about 8 : 1 in reasonable agreement with other estimates (Swann and Magee, 1968; Diaz Gomez et al., 1977). However, after oral administration, this ratio was seen only with doses of 1 mg/kg or more. At doses below this, the ratio rose significantly and at doses of 10 μg/kg it was more than 100 (Fig. 3). As shown in Figure 4, the reason for this increase was that the formation of 7-methylguanine in kidney DNA was much lower after oral administration than after i.v. injection, following doses of 100 μg/kg or less; whereas 7-methylguanine formation in liver DNA was independent of the route of administration.

The simplest explanation of these results is that orally-administered dimethylnitrosamine is taken up into the portal blood supply and is effectively metabolized by the liver in a "first pass effect", thus preventing the exposure of the kidney. Similar results supporting this interpretation were published recently by Diaz Gomez et al. (1977). In this respect, it is of interest that uptake of dialkylnitrosamines from the gastrointestinal tract occurs very rapidly from the upper part of the small intestine with $t_{1/2}$ of less than 3 min for both diethylnitrosamine and dimethylnitrosamine, whereas uptake from the stomach is much slower with $t_{1/2}$ of more than 90 min (Fig. 5). These results are in agreement with those of Heading et al. (1974) and Hashimoto et al. (1976) and suggest that the maximum dose which can effectively be cleared by the liver (which would thus protect other organs) may depend on the rate of gastric emptying of the stomach contents containing the nitrosamine. However, it should be noted that dimethylnitrosamine absorption and uptake into tissues has not been fully investigated. Although the above explanation for the lack of reaction of the kidney with low oral doses of dimethylnitrosamines appears the most probable, it is not proven conclusively. In this respect, it is somewhat unexpected that i.p. injection of low doses of the nitrosamine yields results more similar to those seen with i.v. injection than oral administration (Fig. 4; Pegg and Hui, 1978a). This may be explained by very rapid systemic uptake of the dimethylnitrosamine from the peritoneal cavity without passage through the gastrointestinal circulation. Such uptake would be unusual, but dimethylnitrosamine readily passes through cell membranes (with the exception of the

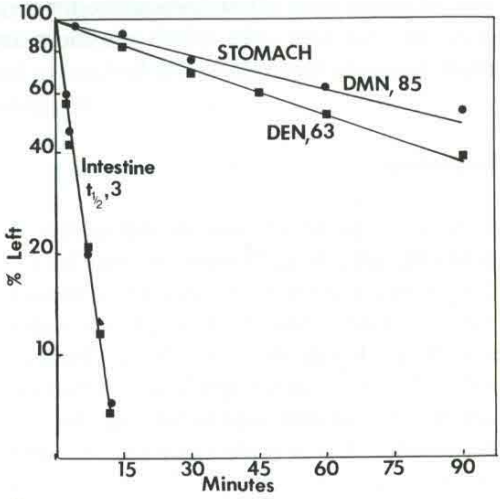

Fig. 5. Uptake of dialkylnitrosamine from stomach or small intestine. Results are shown for the disappearance of dimethylnitrosamine (DMN, ●—●) or diethylnitrosamine (DEN, ■—■) from the stomach or the upper portion of the small intestine. The half time (in minutes) for the uptake is shown on each line. Further details are given under Methods

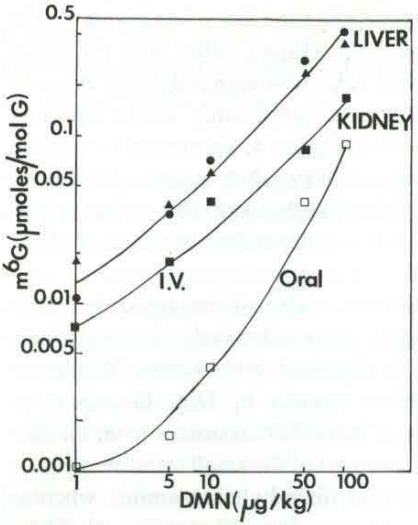

Fig. 6. Presence of O^6-methylguanine in DNA of kidney and liver after intravenous or oral administration of dimethylnitrosamine. Results are shown for O^6-methylguanine (m^6G) present in DNA 6 h after treatment with dimethylnitrosamine by oral administration (□—□, kidney; ▲—▲, liver) or i.v. injection (■—■, kidney, ●—●, liver). Other details were as for Figures 3 and 4

gastric mucosa as described above) and this possibility cannot be ruled out. However, until direct measurements of the nitrosamine concentration in the portal and peripheral circulation after various routes of administration are available, a complete explanation of the reason for the reduced reaction with the kidney after low oral doses cannot be provided.

Whatever the mechanism, the consequences of this phenomenon on O^6-methylguanine levels in the kidney after administration of dimethylnitrosamine via the oral and i.v. routes are quite striking (Fig. 6). When these were measured 6 h after treatment with the nitrosamine, there was only a 2–3 fold difference between levels in the liver and the kidney after i.p. injection because the 8-fold greater formation in the liver was partially compensated for by the more rapid excision in the liver. After oral doses of less than 100 μg/kg the O^6-methylguanine in the kidney was 10 to 50 times less than in the liver because of the much smaller amount of interaction with the kidney. Thus, after oral administration of low doses of the dimethylnitrosamine, virtually all of the reaction was with the liver cells which are most active in catalyzing the removal of O^6-methylguanine from their DNA.

Discussion

There is still controversy as to whether true threshold doses exist for chemical carcinogens (Maugh, 1978). In part, this may be a problem of definition. If a threshold is defined as that dose of a carcinogenic chemical below which no tumors whatsoever would appear whatever the size of the affected population, the concept may never be true and is certainly of little practical value. If a more reasonable definition of a threshold dose as a dose below which no significant increase in tumors within the normal life span of the species is used and 'significant increase' is defined for a given number of affected individuals, there is evidence that such a threshold may occur in experimental animals (Preussmann, 1976; Falk, 1976; Port et al., 1976). If it is accepted, [and as discussed

elsewhere this is by no means proven (Pegg, 1977a)] that the formation and persistence of O^6-methylguanine in DNA provides an initial stimulus towards neoplastic growth, the capacity of the removal system to restore the correct base in DNA might be one factor influencing the threshold. It is shown here that the activity of this system varies with many factors which could, therefore, affect the threshold. Activity also depends on the organ considered (and it is highly probable, although this has not been tested directly that activity differs between different cell types in the same organ). Hence, as demonstrated in several laboratories, the same amount of alkylation damage in one organ may lead to tumors, whereas in others, it does not (Magee et al., 1976; Pegg, 1977a; IARC, 1978). In these studies, several workers have emphasized the resistance of the liver to carcinogenesis by simple dialkylnitrosamines and nitrosamides (Swann and Magee, 1968, 1971; Goth and Rajewsky, 1974; Margison and Kleihues, 1975). This, at first sight, appears paradoxical since the major site of tumor occurrence following prolonged feeding of dimethyl- or diethylnitrosamine is in the liver. However, it must be remembered that as indicated in Figures 3, 5, and 6, after such dietary intake of the carcinogen, virtually all of the metabolism to form the alkylating species occurs in this organ. It is, therefore, conceivable that the metabolism of the dimethylnitrosamine in the liver combined with the high activity for the removal of O^6-methylguanine in this organ protects against carcinogenesis, in general, and particularly in other organs more susceptible to alkylation damage because of a lesser repair ability.

The inhibition of removal of O^6-methylguanine by relatively high doses of dimethylnitrosamine in hamster liver and rat kidney may contribute towards the sensitivities of these organs in which tumors can be induced by large single doses of the carcinogen (Nicoll et al., 1975; Stumpf et al., 1979), but this inhibition may have little relevance to environmental exposure to nitrosamines which occur at much lower doses. However, it should be noted that (a) evidence was obtained for additive effects in retarding O^6-methylguanine excision when rats were exposed to longer chain dialkylnitrosamines prior to treatment with dimethylnitrosamines (Pegg, 1978c) and (b) the dose of dimethylnitrosamine needed for inhibition in the hamster liver was much less than in the rat liver suggesting that other variations with species are also possible.

The degree to which induction of the activity takes place in response to exposure to low levels of the carcinogen is of obvious interest and further work on the extent of this induction, the degree of exposure needed to produce it and the possible induction by other non-carcinogenic agents is in progress. There are interesting similarities between the results obtained in my laboratory showing activity of an enzyme in rat liver which removes O^6-methylguanine from DNA and is inhibited or saturated at high doses of the alkylating agent, but induced by low doses and those obtained by Cairns and collaborators (Samson and Cairns, 1977; Schendel and Robins, 1978; Schendel et al., 1978) showing that an analogous enzyme which protects against mutagenesis is produced by *Escherichia coli* after exposure to alkylating agents.

The importance of growth hormone and of thyroxin in maintaining normal hepatic levels of the enzyme removing O^6-methylguanine from DNA is shown in Table 4, but the mechanism by which these changes are brought about is not yet known. It is highly probable that other nutritional or physiological factors also have effects on the activity of this enzyme system. Its activity, therefore, varies with species, exposure to carcinogens, endocrine factors, and with the organ considered. Although at present it is

not even known whether the enzyme exists in human tissues (and human studies with organs removed at autopsy are urgently needed), it is reasonable to assume that within the genetic and environmental variations of the human population substantial differences in the activity of this enzyme system may exist. If it does contribute towards protection against carcinogenesis by the alkylating carcinogens, individuals having lower activities may be more at risk from these agents. Although it is possible that the 'background' exposure to a wide variety of carcinogenic agents is such that a threshold level is already approached and there is no effectively innocuous additional dose which might be tolerated without increasing cancer incidence (Brown, 1976; Preussmann, 1976), it is known that deficiencies in DNA repair mechanisms are associated with increased risks of cancer from exposure to ultraviolet light or ionizing radiation (Cleaver and Bootsma, 1975; Friedberg et al., 1977). These findings suggest that even though the concept of a threshold dose which would apply to the entire population may be invalid because of widespread genetic variations and differences in 'background' exposures to other carcinogens, it may be possible to identify individuals having greater risks of developing tumors for the same exposure to a carcinogenic stimulus. Although many factors must be considered before such evaluations of risk could be made for chemical carcinogenesis such as nitrosamines and, at present, it is difficult to envisage how the activity of the liver enzyme could be predicted from experiments using those human cells which are widely available, it is possible that in time such evaluations might be accomplished. At present, it can be inferred from the knowledge of the numerous factors affecting the hepatic DNA repair activity for O^6-alkylguanine that predictions based only on average dose exposure may be substantially in error. Because of the complexities of carcinogen activation and DNA repair which provide many steps at which there will be genetic diversity, individual variations in threshold dose are likely to be such that calculations or expectations of overall thresholds are of little value.

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